GUIDANCE[1]

ESTROPIPATE TABLETS:

IN VIVO BIOEQUIVALENCE

AND *IN VITRO* DISSOLUTION

I. INTRODUCTION

A. Clinical Usage

Estropipate is an estrogenic substance prepared from purified crystalline estrone, solubilized as the sulfate and stabilized with piperazine. The oral estropipate tablet is prescribed for the treatment of estrogen deficiency associated with moderate to severe vasomotor symptoms of menopause, atrophic vaginitis, kraurosis vulvae, female hypogonadism, female castration, and primary ovarian failure. The amount of piperazine present in the drug does not exert any pharmacological effect. The drug is administered cyclically, either alone or in conjunction with a progestin. As estrogens have been reported to increase the risk of endometrial carcinoma, the lowest effective dose of estropipate is prescribed and close clinical surveillance is recommended for all women under estrogens therapy (1).

B. Chemistry

The chemical structure of Estropipate appears in the following figure:

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[1]This statement prepared by the Division of Bioequivalence in the Office of Generic Drugs is an informal communication under 21 CFR 10.90 (b) (9) that represents the best judgment of the Division at this time. This statement does not necessarily represent the formal position of the Center for Drug Evaluation and Research, Food and Drug Administration, and does not bind or otherwise obligate the Center for Drug Evaluation and Research, Food and Drug Administration, to the views expressed. For further information about this guidance, contact the FDA Division of Bioequivalence, 7500 Standish Place, Metro Park North, Rockville, MD 20855 (Phone: 301-295-8290); Fax: 301-295-8181).

ESTROPIPATE

Estropipate has a molecular weight of 436.56. The drug is also termed estrone hydrogen sulfate. It is stabilized with piperazine (1:1). Estropipate is water soluble and has no taste or odor.

C. Clinical Pharmacology

Estrogens are steroid hormones, formed mainly by ovarian follicles in premenopausal women and are responsible for the development and maintenance of the female reproductive system and secondary sex characteristics. Estradiol and estrone exist in a reversible equilibrium with estrone sulfate, which may act as an inactive estrogen reservoir for both estrone and estradiol. Of the naturally occurring estrogens, estradiol is the most potent one. Estradiol is converted to estrone by 17ß-dehydrogenase in the liver and other tissues. The conversion is reversible, but estrone formation is favored. Reversible metabolism of estrone to estrone sulfate occurs via sulfurylation in the liver and endometrium (2). Once estradiol is metabolized to estrone by 17ß-dehydrogenase, subsequent steps in degradation are principally oxidative. Estrone either undergoes hydroxylation and then methylation in the A-ring producing methoxyestrone, or it undergoes hydroxylation in the D-ring, producing estriol prior to excretion (3).

In premenopausal women, 95% or more of circulatory estradiol is secreted from the ovary containing the dominant follicle or corpus luteum. Peripheral conversion of estrone to estradiol accounts for most of the remaining estradiol production. Since estradiol is primarily the product of the developing follicle, in the menopause estradiol production is dramatically lowered, and the primary source of estradiol is from the peripheral conversion of estrone (4,5).

D. Pharmacokinetics

Estrone sulfate is absorbed promptly and completely from the gastrointestinal tract. Plasma peak concentration of estrone sulfate occurs at about four hours after oral administration of Estropipate under fasting conditions and at about 5 - 8 hours when taken after meals. The drug is highly bound to serum albumin (6). Most of the estrone sulfate is absorbed in plasma without prior hydrolysis. The reported half life of estrone sulfate is about 12 - 20 hours. The inactivation of the drug takes place mainly in the liver (7 - 10).

II. BIOEQUIVALENCE STUDIES

A. Types of Studies Required

Estropipate is currently available in 0.75, 1.5, 3.0 and 6.0 mg tablets as Ogen ® (Abbott). To gain approval for a generic estropipate tablet, the following studies are required:

1. A single dose, fasting, two way crossover study with the 6 mg strength generic estropipate test product compared to the reference product, Ogen ® 6 mg tablet.

2. *In vitro* dissolution testing for the 6 mg strength.

3. Waivers of bioequivalence study requirements may be granted for the 0.75 mg, 1.5 mg and 3.0 mg strengths if the following conditions are met: a) the sponsor has conducted an acceptable bioequivalence study for its 6 mg strength product as described in this guidance; b) the 0.75 mg, 1.5 mg and 3.0 mg strength products are proportionally similar in their active and inactive ingredients to the 6 mg strength; c) the 0.75 mg, 1.5 mg and 3.0 mg strength products meet an established *in vitro* dissolution specification.

B. Fasting Study

Objective: The objective of this study is to compare the bioavailability of a generic estropipate 6 mg tablet (test product) with that of the reference product Ogen ® 6 mg tablet (Abbott) under fasting conditions.

Design: The study design is a single dose, two treatment, two period, two sequence crossover, with a washout period of at least 7 days. Subjects should be randomly assigned to the two possible dosing sequences.

Facilities: The clinical and analytical sites for the study should be reported, along with the names, titles and the curriculum vitae of the medical, scientific and analytical directors. The starting and ending dates for each clinical study period should be stated. The study protocols should be approved by an institutional review board, and informed consent forms should be signed by all participants.

Subjects: The study should include 24 or more (to ensure adequate statistical results) healthy, postmenopausal females between the ages of 45 to 60 years and within 10% to 15% of the ideal body weight for height and frame size. The postmenopausal status will be based on physiological or surgical menopause of not less than one year confirmed by medical examination. Subjects should be selected on the basis of medical history, physical examination and laboratory tests.

Exclusion Criteria: Subjects with hypersensitivity to the drug or known sensitivity to any of the estrogenic compounds, or a history of hematological, cardiovascular, gastrointestinal, hepatic or renal diseases, drug abuse or alcoholism are to be excluded.

Procedures: The *in vivo* bioequivalence study should be conducted on 6 mg estropipate (5 mg estrone base) tablets of the test and reference (Ogen ^{R}) products. After an overnight (8 to 10 hours) fast, subjects should receive a single dose of the test product or the reference product with 240 ml of water:

Treatment A: Test product, 1x6 mg Estropipate Tablet

Treatment B: Reference product, 1x6 mg Ogen ^{R} (Abbott) Tablet

The test product should be from a production lot or from a lot produced under production conditions. The lot size of the test product should be equal to or more than 100,000. The lot numbers of both the test and reference products and the expiration date for the

reference product should be stated. The potency of the reference product should not differ from that of the test product by more than ± 5%. The sponsor should include a statement of the composition of the test product.

The clinical staff administering the doses should verify that the dose was ingested by each subject. After a one-At least seven days after the last sample collection in the first period of the study, each subject should receive the alternative treatment.

Restrictions: Prior to and during each study phase, subjects should conform to the following restrictions:

a. Subjects should be confined to the clinical facility for 24 hours after dosing and return to the facility for the 30, 36, 48, 60 and 72 hour blood samples.

b. Subjects should be free of all medications including OTC products from two weeks prior to the drug administration until after the study.

c. No alcohol or caffeine containing beverages or food should be consumed for 48 hours prior to the study.

d. Water will be allowed ad libitum except for one hour before and two hours after drug administration.

e. Subjects should be served standardized meals no less than 4 hours after drug administration.

Blood Sampling: Venous blood samples in a volume sufficient for sample analysis should be collected at the following times: -48 hours, -24 hours, 0 hour (immediately preceding drug administration), 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12, 16, 24, 30, 36, 48, 60 and 72 hours post-dose.

Sample Analysis: The plasma should be separated and immediately frozen at -20 ° C until analysis. Plasma samples should be assayed for the estrone and estrone sulfate conjugate. For each subject, the sponsor should state the time elapsed between sample collection and sample assay. An explanation should be provided

for any missing samples.

Analytical Methods: Estrone sulfate and the unconjugated estrone should be extracted from plasma by an efficient solvent extraction procedure (11). The amount of estrone sulfate and unconjugated estrone should be determined by an assay method which is sensitive, specific and reproducible. Radioimmunoassay (12,13) and isotope dilution mass spectrometry (7) methods have been used for the estimation of estrone and estrone sulfate levels in plasma.

The assay methodology should be validated before estimating the plasma estrone and estrone sulfate levels. The standard curves for the drug should be submitted along with a detailed report on the analytical procedure. The final report should include information on the specificity, limit of quantification, linearity, accuracy and precision (interday and intraday) of the assay method. Standards and quality control samples of three different concentrations should be analyzed daily along with the test and reference samples. Baseline plasma concentrations of conjugated and unconjugated estrone at the three sampling times should be averaged to obtain a single baseline value for each of these two compounds. Conjugated and unconjugated estrone concentrations should be provided with and without baseline correction. Samples should be grouped so that all test and references samples for a given subject are assayed on the same day in order to minimize intrasubject variability. The plasma estrone and estrone sulfate data should be summarized for each sampling time with proper standard deviations, coefficients of variation and appropriate statistical analysis (ANOVA).

Pharmacokinetic Analysis: The following pharmacokinetic parameters should be calculated from plasma drug concentration-time data with and without baseline correction:

a. AUC_{0-t}, where T is the last measurable time point calculated by the trapezoidal rule.

b. $AUC_{0-\infty}$, where $AUC_{0-\infty} = AUC_t + C_t/(\lambda_Z)$, C_T is the last measurable drug concentration and λ_Z is the terminal elimination rate constant. $AUC_{0-\infty}$ is

calculated only for baseline-corrected data.

c. C_{max}, equal to peak drug concentration

d. T_{max}, time to peak drug concentration

e. λ_z, the terminal elimination rate constant

Primary data from which these parameters are derived should be included in the submission. Methods of pharmacokinetic calculations should be documented and individual calculations submitted for review. The sponsor should specify which points on the concentration-time curve were used for calculation of the terminal elimination rate constant and provide the correlation coefficient for this determination.

Statistical Analysis: The sponsor should perform analysis of variance (ANOVA) appropriate for a crossover design on the pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} using the General Linear Models (GLM) procedure of SAS or an equivalent program. The statistical model should include terms describing the error attributable to sequence, subjects within sequence [subj (seq)], period and treatment. The sequence effect should be tested against the between subject [subj (seq)] error term. All other main effects should be tested against the residual error from the ANOVA. The ESTIMATE statement in SAS should be used to obtain linear estimates for the adjusted differences between treatment means and the error associated with these differences. The two one-sided tests procedure should be used to calculate 90% confidence intervals about the differences in treatment means for each parameter. The confidence intervals should be expressed relative to the least square mean of the reference product (14).

To determine whether or not the model has adequately adjusted for differences in the baseline estrogen levels of the subjects, an analysis of covariance (ANCOVA) should be conducted using the first baseline value from the study (the single mean baseline value obtained immediately prior to the first dosing period). Terms for baseline and baseline*treatment interaction should be included in the statistical model. If there is no baseline * treatment interaction ($p > 0.05$), the results of the ANOVA can be used for establishing the

90% confidence intervals about the ratio of treatment means [two one-sided tests procedure].

The ANOVA's and ANCOVA's should be conducted on both the original scale and following ln transformation of the treatment data. When a baseline correction is used, the analysis should be conducted prior to log transformation. For the ANCOVA, baseline data should be maintained on the untransformed scale.

Clinical Report: The sponsor should report all clinically adverse reactions that occurred during the study with regard to date and time of onset, duration, frequency, severity and suspected relation to the drug treatment.

III. DISSOLUTION

Dissolution testing should be conducted on 12 estropipate tablets of both the test and the reference products from the lots used in the *in vivo* bioequivalence study. Testing should be conducted utilizing the following conditions:

Apparatus: USP XXII apparatus II (paddle)
Medium: 900 ml of Water (deaerated)at 37 °C
Speed: 75 RPM
Tablets Used: 12
Reference Drug: Ogen ® (Abbott)
Sampling times: 15, 30, 45, and 60 minutes
Method of Estimation: HPLC or any sensitive assay method
Specification: NLT 75% in 60 minutes

For dissolution testing, the sponsor should include the following data:

a. Lot numbers for both test and reference products.

b. The percent dissolution for each dosage unit being tested at each time interval.

c. The mean percent dissolved, the range of percent dissolution and the coefficient of variation for the 12 units being tested at each time interval.

d. Comparative dissolution testing profile data for the test and reference products at 15, 30, 45 and 60 minutes.

e. Validation data for the analytical method used.

f. Expiration date for the reference product.

IV. BIOEQUIVALENCE REQUIREMENTS FOR THE LOWER STRENGTHS OF ESTROPIPATE

To obtain approval for the 0.75 mg, 1.5 mg, and 3.0 mg strength tablets of estropipate, the bioequivalence requirement will be deemed to have been met under either of the following conditions:

1. An acceptable bioequivalence study on the 0.75 mg, 1.5 mg and 3.0 mg tablets, or

2. Evidence of the following conditions:

   a. The 0.75 mg, 1.5 mg, and 3.0 mg tablets are proportionally similar in their active and inactive ingredients to the 6 mg tablets which underwent an acceptable bioequivalence study.

   b. The 0.75 mg, 1.5 mg, and 3.0 mg tablets have satisfactory dissolution characteristics compared to the 6 mg tablets, as well as to the respective dosage strengths of the reference drug product.

V. POTENCY DETERMINATION

Prior to initiation of the bioequivalence study the applicant should determine the potency of the lot of the test drug product to be used in the study. It is recommended that the applicant should ensure that the potency of the lot of the listed product to be used as a reference in the bioequivalence study is within $\pm$ 5% of that for the test drug product. The data on potency should be submitted with the dissolution data.

VI. CONTENT UNIFORMITY TEST

Content uniformity data for 10 test tablets from the lot used in the *in vivo* and *in vitro* testing should be determined and the data submitted along with the dissolution testing data.

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